LC parameters for targeted metabolomic analysis

M1: for amine acids and nucleosides

Amine acids and nucleosides were separated on an Acquity UPLC BEH Amide column (1.7 μm, 2.1mm × 100 mm, Waters). The mobile phase was consisting of acetonitrile-water solution (9:1) as phase A, and acetonitrile-water solution (5:5) as phase B with 10 mM ammonium formate and 1% formic acid as additives in both phases. The binary gradient condition was set as follows: 0-0.5 min at 0% B, 0.5-12.0 min from 0 to 40%, 12.0-15.0 min from 40% to 70%, 15.0-16.0 min at 70%, 16.0-17.0 min from 70% to 0%B with additional 3 min for re-equilibration. The column was kept at 50 °C and the total flow rate was set at 0.3 mL/min.

M2: for bile acids and fatty acids

The separation was performed using a Hypersil GOLD column (1.9 μm, 2.1 mm × 100 mm, Thermo) at a column temperature of 50 °C. The mobile phase consisted of 2 mM ammonium acetate in water (A), and acetonitrile (B). The binary gradient condition was optimized as follows: 0-0.5 min at 17% B, 0.5-12.0 min from 17% to 30%, 12.0-15.5 min from 30% to 55%, 15.5-16.5 min at 55%, and 24.5-27.0 min at 95% with another 3 min for equilibration with total flow rate set at 0.4 mL/min.

M3: for organic acids and carbohydrates

A typical C_{18} column namely ACQUITY UPLC® HSS C_{18} column (1.8 μm, 2.1 mm × 100 mm, Waters) was conducted for the analysis of the derivatives of carbohydrate. The mobile phase consisted of 0.01% formic acid in water (A), and acetonitrile (B). The gradient condition for phase B was as follows: 0-1.0 min at 15%, CTO.RVR value at 0; 1.0-10.0 min at 15%, CTO.RVR value at 1; 16.0-23.0 min from 40% to 95%; 23.0-26.0 min, at 95%, 26.0-27.0 min from 95% to 15% with the column temperature kept at 40 °C. The total flow rate was set at 0.3 mL/min.

M4: for bacteria-derived metabolites

Bacteria-derived metabolites were separated on a PFP C_{18} column (2 μm, 2.1mm × 100 mm, ACE). The mobile phase was consisting of 0.01% formic acid -water solution (phase A) and acetonitrile (phase B). The binary gradient condition was set as follows: 0-1.0 min at 5% B, 1.0-9.0 min from 5 to 75%, 9.0-9.5 min from 75% to 95%, 9.5-12.0 min at 95%, 12.5 min at 5%B with 2.5 min for re-equilibration and the column was maintained at 40 °C. The total flow rate was set at 0.3 mL/min.

M5: for acyl carnitines and lyso-phospholipids

The separation for acyl carnitines and lyso-phospholipids was performed using a PFP C_{18} column (2 μm, 2.1mm × 100 mm, ACE) on the LC-20ADXR HPLC

series system coupled with QTRAP hybrid triple quadrupole mass spectrometer (4500 MD, AB Sciex). The flow rate was set at 0.4 mL/min with the column temperature kept at 50 °C. The mobile phase consisted of water (A) and acetonitrile (B) with 0.1% formic acid as additives in both phases. The binary gradient condition was optimized as follows: 0-0.5 min at 2% B, 0.5-12.0 min from 2% to 98%, 12.0-13.5 min at 98%, 13.60 min at 2% with another 2 min for equilibration.

M6: for lipids

Lipids in different structures were separated on a ACQUITY UPLC® HSS C_{18} column (1.8 μm, 2.1mm × 100 mm, Waters) and detected by QTRAP hybrid triple quadrupole mass spectrometer (4500 MD). Mobile phase A was acetonitrile/water (60/40, V/V) with 10 mM ammonium acetate and mobile phase B was isopropanol/acetonitrile (90/10, V/V) containing 10 mM ammonium acetate. The binary gradient condition was set as follows: 0-2.0 min at 20% B, 2.0-5.0 min from 20 to 70%, 5.0-17.0 min from 70% to 95%, 17.0-17.5 min at 95%, 17.6 min at 20%B with 2.4 min for re-equilibration. The column was kept at 50 °C and the flow rate was set at 0.3 mL/min.